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Tumor necrosis factor enhances low density lipoprotein oxidative modification by monocytes and endothelial cells

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Abstract

The effect of tumor necrosis factor on the oxidative modification of LDL by U937 human monocytes or murine endothelial cells was studied by determination of the lipid peroxidation product content and the electrophoretic mobility of the particle. In the range of concentrations from 2.5 to 10 ng/ml, the cytokine induced a dose-dependent increase in cellular-induced oxidation of LDL. This effect was accompanied by a stimulation of LDL degradation by J774 macrophage-like cells. Concurrently, the TNF-treated cells secreted superoxide anion with a higher rate. Since LDL oxidation is believed to be an inportant feature in the formation of the atherosclerotic plaque, the described effects of TNF might be of importance in long-term exposure to this cytokine during inflammation.

Key words: Tumor necrosis factor; LDL peroxidation; Monocyte; Endothelial cell; Atherosclerosis

1. Introduction

Tumor necrosis factor α (TNF/cachectin) is a monocyte/macrophage-derived cytokine, first identified by its antitumor activity in mice [1]. Besides its cytotoxic/cytostatic activity towards transformed cells in vitro [2,3], TNF exhibits other biological activities on untransformed cells. On monocytes/macrophages, TNF exerts a powerful function on activation and differentiation [4,5], leading to a parasiticidal and bactericidal action. On endothelial cells, TNF induces the release of hematopoietic growth factors [6] and the secretion of interleukins [7,8].

Low density lipoprotein (LDL) is the major cholesterol carrier in plasma, and its oxidative modification by cultured cells such as monocytes [9] or endothelial cells [10] leads to a form which is no longer recognized by the Apo B/E receptor of fibroblasts, but avidly taken up by macrophages via the scavenger receptor pathway [11,12]. This phenomenon is at the origin of cholesteryl-ester accumulation by macrophages and of their subsequent transformation to foam cells located in the atherosclerotic plaque. It is currently believed that oxidative modification of LDL plays an important role in the initiation and progression of atherosclerosis (for review see [13]).

In the present work, we investigated the effects of TNF on the oxidative modification of LDL by monocytes and endothelial cells, as studied by measurement of lipid peroxidation products, and determination of the electrophoretic mobility of the LDL particle. It was demonstrated that the cytokine enhanced LDL modification and superoxide secretion by the two cell types.

2. Materials and methods

2.1. Materials

Human recombinant TNF was from Boehringer Mannheim, Mannheim, Germany. Horse heart cytochrome *c* and horse radish superoxide dismutase were purchased from Sigma, St Louis, MO, USA. Cell culture medium and fetal calf serum were from Gibco, Grand Island, NY, USA. The U937 monocyte-like human cell line and the J774 murine macrophage-like cells were from The American Type Culture Collection, Rockville, Maryland, USA. Na-^{125}I (13–17 Ci/mg) was purchased from Amersham, Buckinghamshire, UK.

2.2. LDL preparation and labelling

LDL (d 1.024–1.050) was prepared from human normal serum by sequential ultracentrifugation according to Havel et al. [14], and dialysed against 0.005 M Tris, 0.05 M NaCl, 0.02% EDTA pH 7.4 for conservation. Prior to oxidation, EDTA was removed by dialysis during 24 h, and LDL, stored at 4°C, were utilized within 2 days. The TBARS value of native LDL was 4–5 nmol MDA/mg LDL protein. ^{125}I-labelling of LDL was performed as described by Bilheimer et al. [15]. The specific activity was about 250 cpm/ng.

2.3. Cell culture

The U937 monocyte-like cells (16) were maintained in suspension in RPMI medium supplemented with 10% inactivated fetal calf serum. Monolayers were obtained when cells were shifted to Ham's F10 medium containing 10^{-7} M 12-*O*-tetradecanoyl phorbol 13-acetate. Experiments were performed with confluent cultures.

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Abbreviations: LDL, low density lipoprotein; TBARS, thiobarbituric acid reactive substances (lipid peroxidation products); TNF, tumor necrosis factor.

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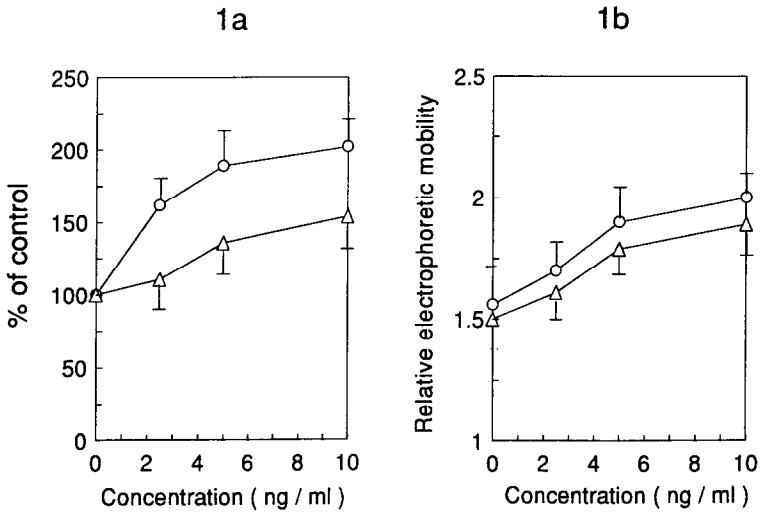

Fig. 1. Effect of TNF on LDL oxidation studied by TBARS production (a), or relative electrophoretic mobility (b). Cells were pretreated for 24 h with TNF before addition of 50 μg/ml LDL protein for a further 24 h incubation time. Means of 6 experimental values ± S.D. 100%: 48 ± 4 nmol equivalent malondialdehyde/mg LDL protein. ○: U937 monocyte cells; △: endothelial cells.

The murine endothelial cell line UNA was a gift from Professor J.D. Chapman, The Fox Chase Cancer Center, Philadelphia, PA, USA. This cell line was characterized by the presence of von Willebrand's factor Cells were maintained in Ham's F10 medium supplemented with 10% fetal calf serum. LDL oxidation was performed on confluent cells.

The J774 macrophage cell line was maintained in suspension in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum. For the determination of LDL degradation, cells were seeded in 3.5 cm Petri dishes (1.5×10^6 cells/dish). All experiments were performed on confluent cultures (about 3×10^6 cells/dish).

2 4. LDL oxidation

Cells in 12-well plates were pretreated for 24 h with TNF in medium supplemented with 0.1% bovine serum albumin before introduction of 50 μg LDL protein/ml for a further 24 h incubation time. The lipid peroxidation products (thiobarbituric acid reactive substances: TBARS) were measured by the fluorometric assay of Yagi [17]. Results are expressed in nmol equivalent malondialdehyde/mg LDL protein, using malondialdehyde from tetramethoxypropane as standard, and calculated as % of control. The modification of the negative net charge of LDL was assessed by agarose gel elctrophoresis at pH 8.6 using a Ciba Corning system.

2.5. LDL degradation by J774 macrophage-like cells

Oxidation of [^{125}I]-LDL by cells in the presence of TNF was first carried out with 20 μg/ml LDL as previously described. The medium was then transferred to J774 cells. LDL degradation was determined after 6 h as described [18], and expressed in ng LDL degraded/mg cellular protein. Blank values from parallel incubations without cells were also determined.

2.6. Secretion of superoxide anion by cells

Cells were treated during 48 h with TNF in medium supplemented with 0.5% bovine serum albumin. Cells were then incubated for 1 h in Dulbecco modified minimum essential medium devoid of phenol red, in the presence of $2 \cdot 10^{-5}$ M cytochrome *c*. Superoxide anion release was calculated from the difference of absorbance at 550 nm in the absence and presence of superoxide dismutase 100 μg/ml, using a molar extinction coefficient of 21/mM/cm.

Each experiment was performed at least 2 times in duplicate. Statistical analysis was performed by the Student's *t*-test.

3. Results

The effect of TNF on LDL oxidative modification by monocytes and endothelial cells was first investigated by determination of the lipid peroxidation product (TBARS) content and of the electrophoretic mobility of the particle. As it can be seen in Fig. 1a, at concentrations ranging from 2.5 to 5 ng/ml, TNF increased in a dose-dependent manner the TBARS content and the electrophoretic mobility of LDL exposed to either monocytes or endothelial cells. The U937 monocyte cell line was more sensitive to the action of TNF, in terms of TBARS production, than UNA endothelial cells. At 10 ng/ml, the cytokine induced a 2- and 1.5-fold increase in TBARS content of LDL modified by monocytes and endothelial cells, respectively. Concomitantly, the relative electrophoretic mobility was increased in a parallel manner in both cell types (Figs. 1b and 2). Furthermore, conjugated dienes determination by absorption at 234 nm also confirmed the effect of TNF: at 10 ng/ml, an increase of 40–50% and 15–25% was observed for monocytes and endothelial cells, respectively.

Table 1
Degradation by J774 macrophages of [^{125}I]LDL oxidized by monocytes or endothelial cells treated with TNF

Addition	[^{125}I]LDL degradation (ng/mg protein)	
Without cells or Cu^{2+}	137 ± 11	
	Monocytes	Endothelial cells
None	436 ± 37	574 ± 42
TNF 2.5 ng/ml	532 ± 42*	644 ± 63
TNF 5 ng/ml	574 ± 43**	679 ± 54*
TNF 10 ng/ml	603 ± 61***	709 ± 67**

Oxidation of [^{125}I]LDL (20 μg/ml) by monocytes and endothelial cells was carried out as described in Fig. 1 legend. The medium was then transferred to J774 macrophages for determination of LDL degradation after 6 h incubation at 37°C. Results are expressed in ng LDL degraded/mg cellular protein. Means of 4 experimental values ± S.D. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ by the Student's *t*-test.

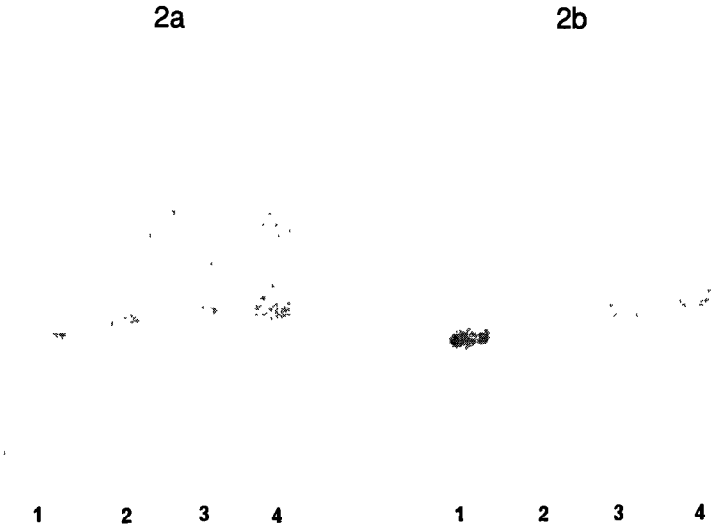

Fig. 2. Effect of TNF on LDL oxidation by monocytes (a) or endothelial cells (b), studied by electrophoretic mobility. LDL oxidation was performed as described in the legend of Fig. 1. Lane 1, native LDL; lane 2, oxidized LDL in the absence of TNF; Lane 3, oxidized LDL in the presence of 5 ng/ml TNF; lane 4, oxidized LDL in the presence of 10 ng/ml TNF.

Since it is well known that LDL oxidation leads to a form rapidly taken up and degraded by macrophages, the action of TNF on cell-induced modification of LDL was also assessed by the subsequent step of LDL degradation by macrophages. From data given in Tabel 1, it can be noted that LDL degradation was approximately 3-fold increased after cellular modification by non-treated cells. In addition, when the oxidation was conducted with TNF-treated cells, the LDL degradation was enhanced in a dose-dependent manner. The effect of TNF was again more marked with monocyte-like cells: at 10 ng/ml, TNF enhanced by 55% and 30% the LDL degradation after oxidation by monocytes or endothelial cells, respectively. These results are in close accordance with those presented in Fig 1a, concerning the TBARS production during LDL oxidation.

In the next experiment, we tested whether the stimulation of LDL modification by TNF was related to a parallel increase in superoxide anion release by cells. It can be concluded from the data presented in Table 2 that the secretion of superoxide anion was enhanced by TNF. At 10 ng/ml, the cytokine induced a 1.5- and 1.3-fold increase in superoxide production by monocytes and endothelial cells, respectively.

4. Discussion

The cytokine TNF significantly stimulated LDL oxidative modification by monocytes or endothelial cells. This effect was accompanied by enhanced degradation of the LDL particle by macrophages. It should be noted that when LDL oxidation was conducted using copper ions instead of cultured cells, no effect of the cytokine could be evidenced (results not shown). This indicates that most probably TNF did not act on the LDL particle itself, but rather on the cellular mechanisms involved in LDL oxidative modification. In this regard, the difference in sensitivity to TNF between the two studied cell types might be due to the different mechanisms whereby cultured cells promote LDL oxidative modification. It has been suggested that the ability of a given cell type to oxidize LDL was correlated to the rate of superoxide anion secretion [19]. The role of superoxide anion in the initiation of LDL oxidation was further evidenced by the fact that the enzyme superoxide dismutase can inhibit LDL modification by monocytes/macrophages [20,21]. However, in the case of human endothelial cells, superoxide dismutase can only partially prevent LDL oxidation [19]. In our system, superoxide dismutase partially blocked LDL modification by cells by approximately 20–25% (data not shown). Furthermore, even in the presence of the enzyme, the stimulatory effect of TNF was

Table 2
Effect of TNF on superoxide anion release by monocytes and endothelial cells

Addition	Superoxide anion release (nmol/h/mg protein)	
	Monocytes	Endothelial cells
None	166 ± 17	134 ± 12
TNF 2.5 ng/ml	197 ± 16	147 ± 11
TNF 5 ng/ml	209 ± 18*	172 ± 13*
TNF 10 ng/ml	252 ± 21**	199 ± 18**

Monocytes or endothelial cells were treated with TNF during 48 h in medium supplemented with 0.1% bovine serum albumin before determination of the superoxide anion release. Means of 4 experimental values ± S.D. *$P < 0.05$; **$P < 0.01$ by the Student's t-test.

still observed, thus indicating that TNF also acts through other mechanisms distinct from superoxide anion secretion. It has been reported that LDL modification by endothelial cells also involves a superoxide-independent pathway, the lipoxygenase pathway [22]. In this regard, the hypothesis of a positive regulation of the lipoxygenase activity by TNF might be raised. It is of interest that TNF has been demonstrated to activate the synthesis of cyclooxygenase [23], another enzyme involved in the metabolism of polyunsaturated fatty acids.

Our studies also showed that in the same range of concentrations, TNF stimulated superoxide anion release by the studied cells. This result is in accordance with the well known effect of the cytokine on monocytes/macrophages activation and differentiation. The stimulatory action of TNF on the release of active oxygen species and of reactive nitrogen has been reported [4,5]. In addition, it was demonstrated that TNF is a powerful inducer of hydroxyl radical production [24] or superoxide anion secretion [25]. This effect is currently believed to be responsible for the cytotoxic effect of TNF on cancer cells [26]. However, it must be stressed that under our experimental conditions, no cytotoxicity was noticed in TNF-treated cells, as assessed by cellular adherence and thymidine incorporation (data not shown).

Previous studies pointed at the multiple effects of TNF on endothelial cell functions. In endothelial cells, TNF induces the expression of mononuclear leukocyte adhesion molecule [27]. The cytokine also strongly promotes tissue factor-like procoagulant activity [28] and suppresses endothelial cell surface anticoagulant activity [29]. A chemotactic action of TNF towards monocytes was already reported [30]. It should be noted that the concentrations of TNF utilized in the current studies are within the physiologic range [31]. It can thus be suggested that TNF might exert a non-negligible effect on the initiation and progression of the atherosclerotic plaque.

The production of TNF by activated monocytes, macrophages and T lymphocytes has an evident protective role against bacterial and parasitic infections. However, long-term exposure to this cytokine might induce some harmful action rising from the numerous side effects of TNF on endothelial cell functions, especially concerning the formation of atheromatous lesions.

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